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GENETIC VARIABILITY IN DEEP-SEA ORGANISMS

RODOLFO COSTA AND PAOLO MARIA BISOL

Istituto di Biologia Animale, Università di Padova, Via Loredan 10, Padova; and Istituto di Biologia del Mare, C.N.R., Riva 7 Martiri 1364/A, Venezia, Italy

In the last few years many researchers have studied genetic variability in marine organisms, in a search for correlations between polymorphism and environmental stability. A high degree of polymorphism has been found in deep-sea organisms which indicates a high genetic variability (Schopf and Gooch, 1971; Doyle, 1972; Gooch and Schopf, 1972; Ayala and Valentine, 1974; Ayala, Valentine, Hedgecock and Barr, 1975). These data are not in agreement with the hypothesis that predicted an impoverishment of genetic variability in those populations that live in highly specialized environments (Levins, 1968; Grassle, 1972).

In these works a highly stable deep environment had always been chosen; in the present research, however, the species considered come from an area that presents a certain instability of some environmental features. This area is the Wyville-Thomson Ridge (Shetland-Faroe Channel) which is intermittently subjected to overflows of Norwegian Sea deep waters flowing in a southwest direction towards the Northern Rockall Trough (Ellet and Roberts, 1973).

Materials and Methods

Populations studied

Samples were collected in the area of the Northern Rockall Trough and the Wyville-Thomson Ridge (Northeast Atlantic Ocean) in the period from June 30, 1976 to July 13, 1976, during the scientific cruise made by the R.R.S. CHALLENGER, organized by the Scottish Marine Biological Association. The Agassiz-trawl was used for the sampling. On July 2 specimens of the crustacean species, *Munidopsis hamata*, were collected at a depth of 1331 meters along a transect from 59° 13′ 06″ N; 08° 01′ 05″ W to 59° 13′ 46″ N; 08° 00′ 58″ W. On July 7 gastropods of a species belonging to the genus *Buccinum* and echinoderms belonging to the species *Ophioglypha bullata* were collected at a depth of 1058 m on a transect extending from 60° 04′ 08″ N; 05° 56′ 80″ W to 60° 04′ 86″ N; 05° 59′ 02″ W. On July 11 the echinoderm *Ophiomusium lymani* was collected at the depth of 1900 m from 57° 06′ 08″ N; 12° 06′ 01″ W to 57° 06′ 73″ N; 12° 05′ 27″ W.

The fishing operation lasted about four hours on July 2 and 7 and about six hours on July 11. Specimens were immediately stored in the ship's deep freezer at −20° C, and in the laboratory the specimens were stored at −30° C until the time of dissection.

Gel preparation

The starch used is Sigma (Sigma Chemical Co.). A 12% (w/v) solution of starch in gel buffer was heated to near boiling point and degassed with an aspira-

tor and then poured in a plexiglass mold, 10 × 20 × 0.3 cm (volume 100 ml). After the electrophoresis, for each run, two different stains were carried out by dividing the gel in two layers. In some cases electrophoresis was performed on acrylamide gel (from Serva feinbiochemica) on vertical slab. The methods followed in these cases have been described by Bisol, Varotto and Battaglia (1976).

Gel and electrode buffers

Six types of buffer were used (the buffers for the gel were the same used for the electrodes, diluted 1 : 1, except the buffer D diluted 1 : 10) : A, 0.083 M Tris and 0.081 M boric acid, pH 8.5; B, 0.083 M Tris and 0.048 M boric acid, pH 8.8; C, 0.01 M Tris, 0.01 M maleic acid, 0.0025 M NaOH and 0.0002 M $MgCl_2$, pH 7.4; D, 0.135 M Tris and 0.045 M citric acid, pH 7; E, 0.023 M Tris and 0.005 M citric acid, pH 8; and F, 0.076 M Tris and 0.05 M citric acid, pH 8.8.

Sample preparation

Crude protein extracts were prepared from dissected samples of tissue (soft tissue of ophiuroids, muscle and hepatopancreas of decapods, foot and visceral sac of the gastropods) by homogenization in about two volumes of Tris-HCl pH 8, 0.05 M buffer. The homogenates were centrifugated at 15,000 rpm for 10 min. Five to 10 μl of the supernatant were absorbed with a 4 × 4 mm piece of Whatman

TABLE I

Enzymes assayed and buffer systems used in the study of genetic variation in four species of deep-sea organisms.

Enzymes	Abbreviation	Buffer system*	Number of loci scored in			
			Buccinum sp.	*Ophioglypha bullata*	*Munidopsis hamata*	*Ophiomusium lymani*
Acid phosphatase	*Acph*	A	3	2	2	1
Alkaline phosphatase	*Aph*	B	7	1	1	2
Aminopeptidase	*Ap*	A	3	2	3	2
Esterase	*Est*	A	6	7	10	4
Glyceraldehyde-3-phosphate dehydrogenase	*G-3-pdh*	F	—	1	—	—
Hexokinase	*Hk*	D	—	1	1	1
Isocitrate dehydrogenase	*Idh*	C	1	1	1	1
Leucine aminopeptidase	*Lap*	A	2	2	1	2
Lactate dehydrogenase	*Ldh*	A, C	1	2	1	1
Malate dehydrogenase	*Mdh*	A	1	1	1	1
Malic enzyme	*Me*	E, F	1	—	1	1
Nothing dehydrogenase	*Ndh*	A	2	—	1	—
Phosphoglucomutase	*Pgm*	A, C	1	1	1	1
Phosphohexose isomerase	*Phi*	A, G	1	1	1	1
6-Phosphogluconate dehydrogenase	*6-Pgdh*	D	—	—	1	—
Tetrazolium oxidase	*To*	A, E	—	3	3	1

* See Materials and Methods for a description of the buffer systems.

TABLE II

Allelic frequencies at eleven polymorphic loci in a deep-sea population of Buccinum *sp.*

Locus	N*	Number of alleles	Allelic frequencies			Frequency of heterozygotes		Polymorphic loci**	
			a	b	c	Observed	Expected	(1)	(2)
Ap-3	44	3	0.045	0.932	0.023	0.136	0.129	+	+
Aph-4	44	3	0.250	0.045	0.705	0.227	0.439	+	+
Aph-5	44	3	0.273	0.636	0.091	0.500	0.512	+	+
Aph-6	44	3	0.114	0.636	0.250	0.636	0.520	+	+
Est-1	44	2	0.159	0.841	—	0.318	0.268	+	+
Idh-1	44	2	0.023	0.977	—	0.045	0.044	+	−
Lap-2	44	3	0.341	0.568	0.091	0.318	0.553	+	+
Mdh-1	44	2	0.886	0.114	—	0.136	0.201	+	+
Pgm-1	44	3	0.023	0.955	0.022	0.091	0.088	+	−
Phi-1	44	2	0.023	0.977	—	0.045	0.044	+	−
Me-1	44	2	0.068	0.932	—	0.136	0.127	+	+

* N: number of genes (twice number of individuals) sampled at each locus.

** Two criteria are used to decide whether a locus is polymorphic, on the basis of the frequency (P) of the most common allele: (1) $P < 0.990$; (2) $P < 0.950$. The same criterion was used in Tables III, IV, and V.

No. 3 filter paper. The soaked filter paper wicks were placed into the starch gel slab for horizontal electrophoresis. All these operations were carried out in a cold room at 4° C.

Enzyme assays

The systems were stained according to Shaw and Prasad (1970), Ayala, Powell, Tracey, Murão, and Pérez-Salas (1972) and Bisol (1976), with some modifications concerning, almost exclusively, the pH of stain buffers.

Gel fixation

After the enzyme bands appeared, the reaction was stopped by washing the gel with water and adding fixing solution: 50 parts methanol, 10 parts glacial acetic acid, and 40 parts H_2O. After fixation, the electrophoretic patterns were ready for interpretation.

RESULTS

The observed patterns have been interpreted under the assumption of Mendelian segregation in diploids (Brewer, 1970). Frequencies have been calculated on the basis of the Hardy-Weinberg principle. The nonvarying zones have been assumed to be controlled by a single locus.

Buccinum sp.

The electrophoretic analysis performed on 22 specimens of this snail for 12 enzymatic systems has revealed 29 zones of activity (Table I).

TABLE III

Allelic frequencies at thirteen polymorphic loci in a deep-sea population of Ophioglypha bullata.

Locus	N	Number of alleles	Allelic frequencies			Frequency of heterozygotes		Polymorphic loci	
			a	b	c	Observed	Expected	(1)	(2)
Ap-2	50	2	0.460	0.540	—	0.200	0.497	+	+
Aph-1	48	2	0.729	0.271	—	0.208	0.400	+	+
Est-4	42	2	0.333	0.667	—	0.286	0.444	+	+
Est-6	44	3	0.023	0.409	0.568	0.227	0.509	+	+
Hk-1	44	3	0.045	0.500	0.455	0.591	0.541	+	+
Idh-1	50	2	0.980	0.020	—	0.040	0.039	+	−
Lap-1	38	2	0.158	0.842	—	0.211	0.266	+	+
Lap-2	38	2	0.763	0.237	—	0.158	0.361	+	+
Ldh-2	68	3	0.059	0.676	0.265	0.147	0.469	+	+
Mdh-1	50	3	0.060	0.700	0.240	0.280	0.224	+	+
Pgm-1	50	2	0.640	0.360	—	0.240	0.460	+	+
Phi-1	50	2	0.640	0.360	—	0.240	0.460	+	+
To-3	50	2	0.600	0.400	—	0.320	0.480	+	+

One of these zones, leucine aminopeptidase, has not been included in the data, as it was found in only a few specimens. It did, however, show different electromorphs. Among the remaining 28 zones, 17 appeared monomorphic, while 11 were polymorphic (Table II).

Ophioglypha bullata

Twenty-five individuals of this brittle star were analyzed. Nine additional individuals have been analyzed subsequently so as to be able to interpret the lactate dehydrogenase system. Twenty-three activity zones attributable to the action of of 23 genic loci were found (Table I). Two further esterase zones, being characterized by an electrophoretic pattern of uncertain interpretation, have been omitted in the data (Table III).

TABLE IV

Allelic frequencies at seven polymorphic loci in a deep-sea population of Munidopsis hamata.

Locus	N	Number of alleles	Allelic frequencies				Frequency of heterozygotes		Polymorphic loci	
			a	b	c	d	Observed	Expected	(1)	(2)
Ap-3	44	2	0.386	0.614	—	—	0.409	0.474	+	+
Est-9	28	2	0.964	0.036	—	—	0.071	0.068	+	−
Hk-1	46	4	0.043	0.783	0.152	0.022	0.348	0.362	+	+
Idh-2	44	2	0.159	0.841	—	—	0.136	0.267	+	+
Lap-3	42	3	0.524	0.452	0.024	—	0.714	0.520	+	+
Pgm-1	38	3	0.850	0.075	0.075	—	0.100	0.266	+	+
6-Pgdh	46	2	0.696	0.304	—	—	0.435	0.423	+	+

TABLE V

Allelic frequencies at nine polymorphic loci in a deep-sea population of Ophiomusium lymani.

Locus	N	Number of alleles	Allelic frequencies					Frequencies of heterozygotes		Polymorphic loci	
			a	b	c	d	e	Observed	Expected	(1)	(2)
Ap-1	94	2	0.138	0.862	—	—	—	0.277	0.238	+	+
Ap-2	94	2	0.745	0.255	—	—	—	0.170	0.380	+	+
Hk-1	88	5	0.080	0.364	0.227	0.284	0.045	0.886	0.727	+	+
Idh-1	68	2	0.353	0.647	—	—	—	0.235	0.457	+	+
Ldh-1	92	3	0.826	0.152	0.022	—	—	0.261	0.294	+	+
Me-1	82	3	0.183	0.756	0.061	—	—	0.195	0.391	+	+
Pgm-1	92	5	0.087	0.185	0.413	0.283	0.033	0.543	0.701	+	+
To-1	92	4	0.054	0.402	0.261	0.283	—	0.652	0.687	+	+
Phi-1	94	2	0.926	0.074	—	—	—	0.021	0.138	+	+

Munidopsis hamata

Among the 29 zones of activity detected by staining for 15 enzymatic systems (Table I), 28 have been interpreted, while one esterase has been omitted. The data concerning the polymorphic systems are reported in Table IV. To clarify the complex pattern of esterases (ten activity zones of which eight are monomorphic), the heart, hepatopancreas, eye, and muscle were analyzed separately. The various loci showed distributions excluding overlaps of two distinct loci in the patterns resulting from each individual *in toto*. The 6-phosphogluconate dehydrogenase was obtained as secondary stain of the hexokinase (unpublished data).

Ophiomusium lymani

Only 13 of the 15 stains gave positive results (Table I). Among 19 detected zones, two leucine aminopeptidases were difficult to interpret and were not utilized

TABLE VI

Summary of genetic variation in four deep-sea species of marine animals.

Parameter	*Buccinum* sp.	*Ophioglypha bullata*	*Munidopsis hamata*	*Ophiomusium lymani*
Number of loci	29	24	29	17
Number of individuals	22	25	23	47
Genes sampled per locus	44	48.261 ± 1.221	44.857 ± 0.678	90.705 ± 1.626
Alleles per locus	1.586 ± 0.153	1.739 ± 0.157	1.379 ± 0.144	2.118 ± 0.341
Polymorphic loci	0.357	0.565	0.250	0.529
Polymorphic loci with $P < 0.95$	0.250	0.521	0.214	0.529
Frequency of heterozygotes:				
average over individuals	0.093 ± 0.008	0.122 ± 0.019	0.075 ± 0.006	0.189 ± 0.012
average over loci (obs.)	0.092 ± 0.031	0.137 ± 0.032	0.079 ± 0.033	0.191 ± 0.065
average over loci (exp.)	0.104 ± 0.034	0.224 ± 0.047	0.085 ± 0.031	0.236 ± 0.067

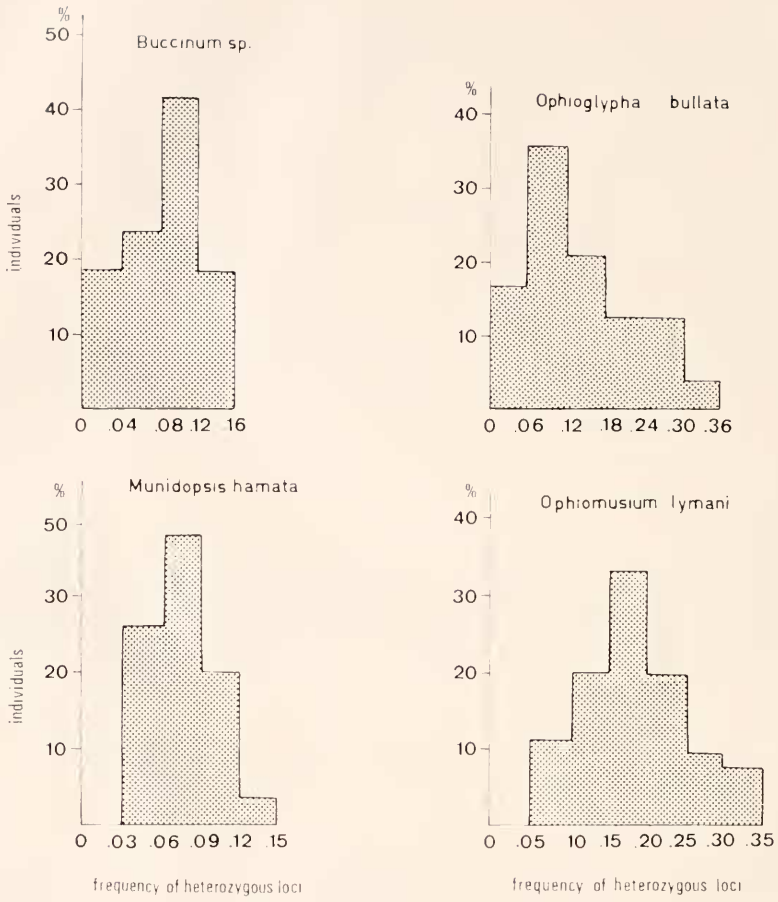

FIGURE 1. Distribution of the heterozygosities of individuals.

for the estimation of the genetic variability of this species (Table V) even though, in all probability, they were the expression of polymorphic loci as reported for a Pacific Ocean population (Ayala and Valentine, 1974).

In Figure 1 and 2 are reported the distributions of heterozygotes both for individuals and for loci. The summary data concerning the degree of genetic variability of the four species are given in Table VI.

DISCUSSION

An intermittent overflow of dense and deep waters, coming from the Norwegian Sea, flows into Rockall Trough, a depression of the Wyville- Thomson Ridge (Ellet and Roberts, 1973). Such overflow was observed and studied during the period August 24–29, 1973, by the R.R.S. CHALLENGER (Currie, Edwards and Ellet, 1974), along the western side of the Wyville-Thomson Ridge. Geological data indicate

that sediments from these intermittent "overspills" can be found all along the extension of the Wyville-Thomson Ridge. This overflow is the source of the dense water in the deepest point of the Rockall Trough, and, moreover, it causes periodical variations in salinity, density and temperature (Ellet and Martin, 1973).

Our results indicate that, for the species *Munidopsis hamata* and *Buccinum* sp., the agreement of the observed distribution of genotypes with the Hardy-Weinberg is, in general, satisfied. This is not the case with several loci of the two ophiuroids, where a significant deficiency of heterozygotes appears to be the rule. In our opinion this fact should not be sufficient to invalidate the kind of genetic control postulated for the observed patterns. Artifacts due to a poor storage of the material, as well as erroneous interpretations due to between-loci hybridizations (most loci taken into account showed a single zone of reaction) can reasonably be discarded.

Moreover, previous authors, such as Doyle (1972) for an esterase locus, and Murphy, Rowe, and Haedrich (1976) for many loci, observed a significant deficiency of heterozygotes in Atlantic populations of *Ophiomusium lymani* and other ophiuroids.

Our data partially confirm the high degree of genetic variability found by other authors in deep-sea organisms. In the ophiuroid, *Ophiomusium lymani,* the esti-

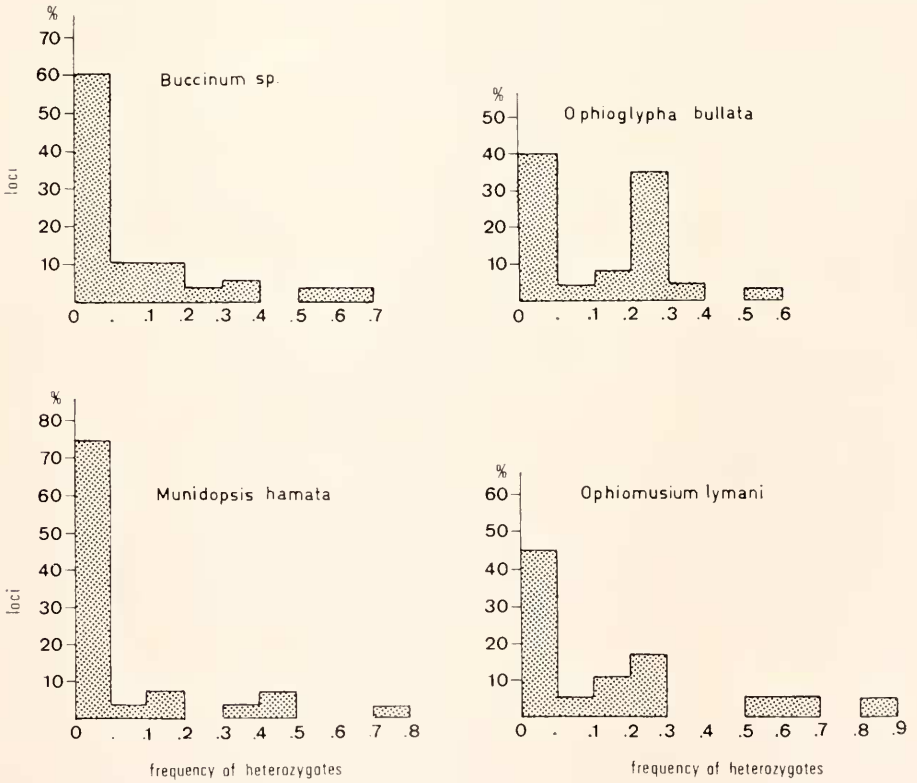

FIGURE 2. Distribution of loci relative to the proportion of heterozygous individuals at each locus.

mated frequency of the polymorphic loci, equal to 0.529, is very near to that found by Ayala in a population of the same species from the Pacific Ocean (Ayala and Valentine, 1974). In the other ophiuroid, *Ophioglypha bullata,* the frequency of polymorphic loci is 0.521. The values in the gastropod, *Buccinum* sp., and in the crustacean, *Munidopsis hamata,* 0.250 and 0.214, respectively, are clearly lower. We believe that this lower variability is not the result of a misinterpretation of the electrophoretic patterns (*i.e.,* esterases). The use of competing substrates which give differential stains, and the electrophoretic analysis of homogenates of single organ parts, supports the assumption that each apparently unvarying zone is the expression of a single locus.

The different levels of genetic variability cannot be related to the degree of environmental stability. The deep-sea organisms affected by the overspills are *Munidopsis hamata* (low variability) and *Ophiomusium lymani* (high variability), while the species *Ophioglypha bullata* (high variability) and *Buccinum* sp. (low variability) have been sampled northeast of the Wyville-Thomson Ridge in an area not affected by these intermittent overspills. This may indirectly indicate that the selective role of the environmental physical parameters is nil or very small. On the other hand, the overall genetic variability is an adequate indication of the action of selection, which can be found only in extended and periodical samplings such as to demonstrate significant variations of genic frequencies.

Ayala *et al.* (1975) hypothesized that genetic variability in deep-sea populations could be maintained by selective pressures of environmental biotic factors. With our data it is impossible either to deny or to confirm such an hypothesis. Nevertheless, from the analysis of the results, a significant consideration emerges: the different levels of variability in organisms belonging to different phyla could mean that the adaptive strategies of the species are different. It has been shown that in certain echinoderms a greater variability is found in the species from a deep-sea environment, as compared to those from shallow water (Schopf and Murphy, 1973). In the decapod, *Munidopsis hamata,* on the other hand, polymorphism is similar to that of other species of decapods coming from different environments (Hedgecock, in Valentine, 1976). The strategies of adaptation are the result of interactions between environmental factors and biological characteristics of the species, such as the life cycle, reproductive type and population size.

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Summary

Four species of deep-sea marine invertebrates, coming from the vicinity of the Rockall Trough (Shetland-Faroe Channel), have been studied by means of gel electrophoresis. The degree of genetic polymorphism found would seem to indicate

absence of correlation with the particular environmental factors which characterize the sampled area. The results suggest that the adaptive strategies of organisms belonging to different phyla are different.

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